

Effector CD8⁺ T cell-derived interleukin-10 enhances acute liver immunopathology

Jessica Fioravanti, Pietro Di Lucia, Diletta Magini, Federica Moalli, Carolina Boni, Alexandre Pierre Benechet, Valeria Fumagalli, Donato Inverso, Andrea Vecchi, Amleto Fiocchi, Stefan Wieland, Robert Purcell, Carlo Ferrari, Francis V. Chisari, Luca G. Guidotti, Matteo Iannacone

Table of contents

Fig. S1.....	2
Fig. S2.....	3
Fig. S3.....	4
Fig. S4.....	5
Fig. S5.....	6
Fig. S6.....	7

Figure S1

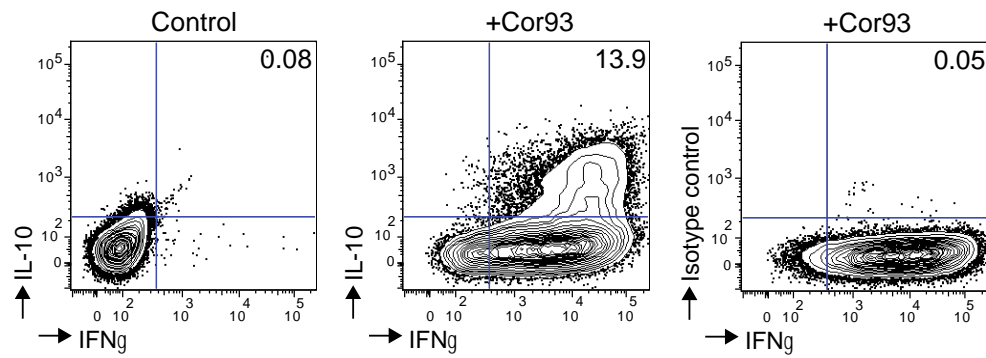


Fig. S1. *Cor93 CD8 T_E produce IL-10 upon Ag recognition.* Cor93 CD8 T_E were incubated with PBS (control) or with Cor93 peptide (+Cor93). Five hours later cells were assessed for their capacity to produce IFN-γ and IL-10 by flow cytometry. Numbers represent the percent of cells within the gate. The right plot shows a staining with an isotype control in place of the anti-IL10 antibody. Results are representative of 3 independent experiments.

Figure S2

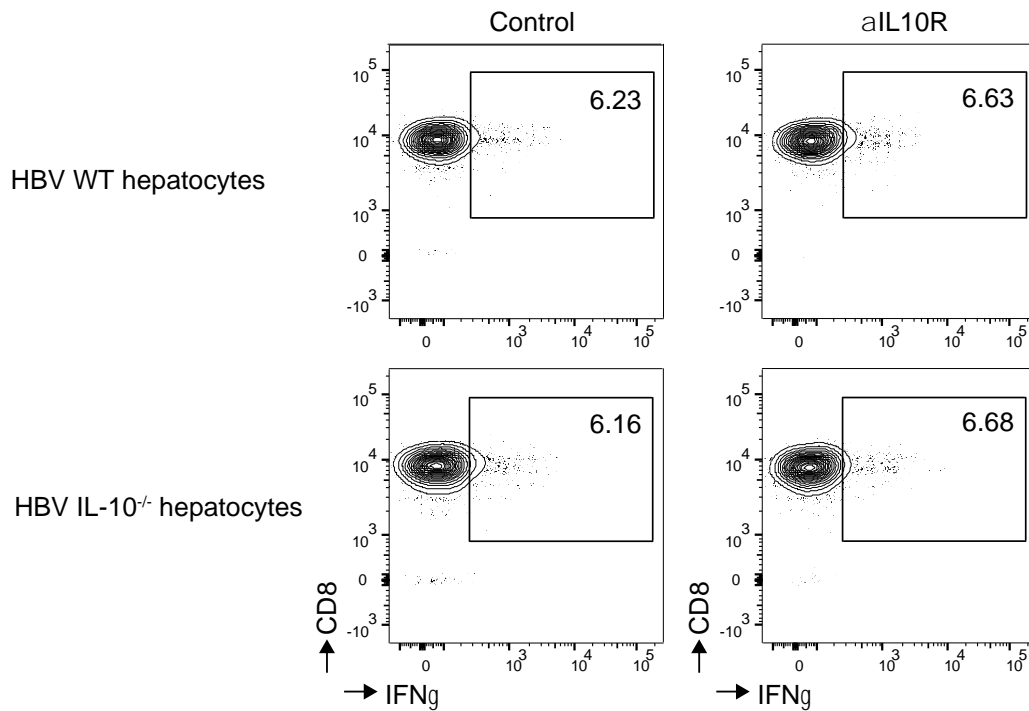


Fig. S2. *IL-10 does not affect hepatocellular Ag presentation.*

Hepatocytes isolated from IL-10^{+/+} (WT, top) or IL-10^{-/-} (bottom) HBV replication-competent transgenic mice were incubated with Cor93 CD8 T_E in the presence (α IL10R, right) or absence (Control, left) of anti-IL10R α Abs (1 μ g/ml). Five hours later Cor93 CD8 T_E were assessed for their capacity to produce IFN- γ by flow cytometry. Numbers represent the percent of cells within the gate. Results are representative of 2 independent experiments.

Figure S3

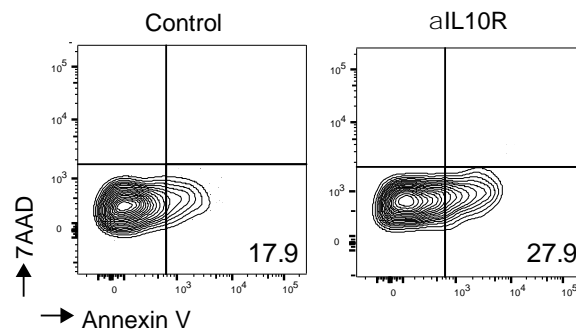


Fig. S3. Representative flow cytometry plots and gating strategy utilized for determining CD8 T_E apoptosis. Representative flow cytometry plots and gating strategy utilized for the analysis of Fig. 2E. Numbers represent the percentage of cells within the gate.

Figure S4

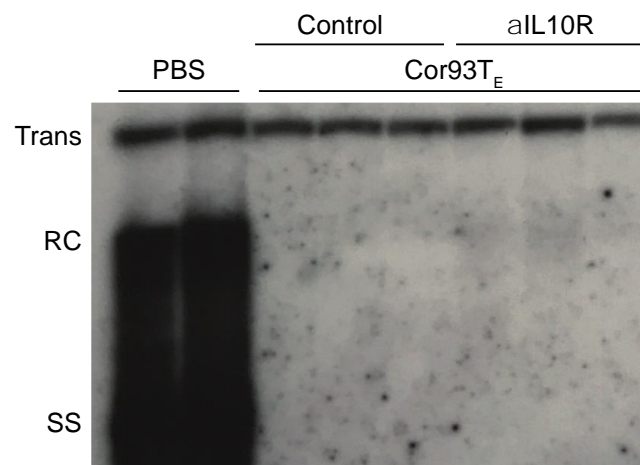


Fig. S4. *Blocking IL-10 receptor signaling does not affect the antiviral potential of Cor93 CD8 T_E.* Total hepatic DNA was isolated from 2 HBV replication-competent transgenic mice treated with PBS and from 6 HBV replication-competent transgenic mice treated (α IL10R) or not (Control) with anti-IL10R α and killed 2 days after the injection of 10^7 Cor93 CD8 T_E. Hepatic DNA was assessed for HBV replication by Southern blot analysis. Trans, integrated transgene; RC, relaxed circular DNA; SS, single stranded DNA.

Figure S5

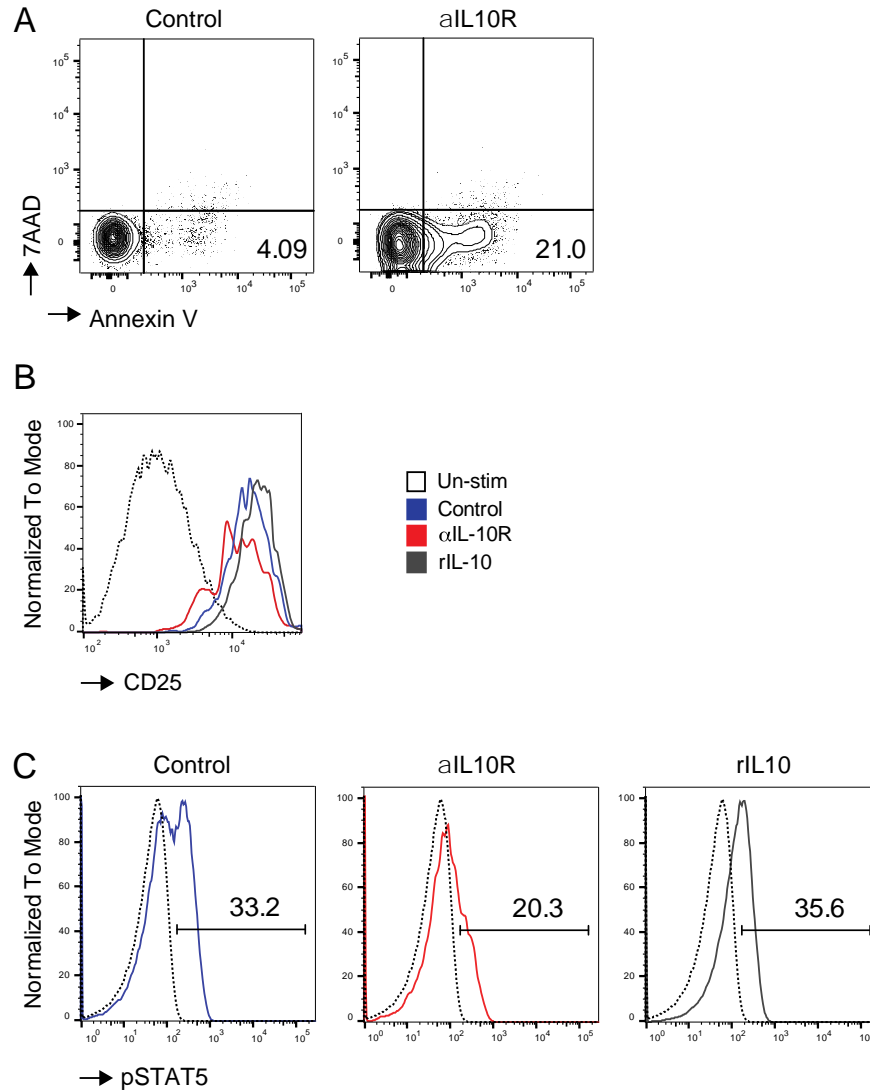


Fig. S5. Representative flow cytometry plots and gating strategy utilized for determining CD8 T_E apoptosis, CD25 staining and pSTAT5 staining. **(A-C)** Representative flow cytometry plots and gating strategy utilized for the analysis of Fig. 3A **(A)**, Fig. 3B **(B)** and Fig. 3C **(C)**. Numbers represent the percentage of cells within the gate.

Figure S6

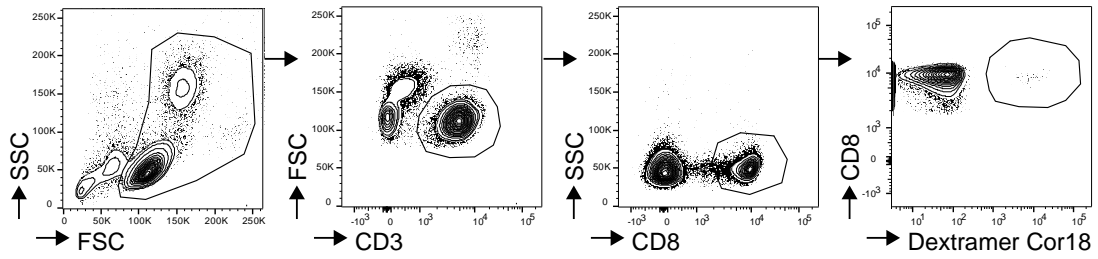


Fig. S6. *Gating strategy for HBV dextramer⁺ CD8⁺ T cells.*

Flow cytometry gating strategy utilized for the analysis of dextramer Cor18⁺ cells described in Fig. 3D.